

CYTOMAGNETOMETRIC MEASUREMENT OF THE ENERGY OF THE INTRACELLULAR MOVEMENTS.

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Abstract—Magnetic particles were given to macrophage-like cultured cells J774A.1 to perform cytomagnetometry in which the magnetic field from the cells was measured and the mechanical parameters of the cytoplasm and the energy E_r of the intracellular movement were estimated. The response of the cells to ATP-synthesis inhibitor MIA measured by cytomagnetometry was intriguing; the energy E_r became larger contrary to our expectation. We have been searching for the reason for this phenomenon by the immunofluorescent microscopy and the use of other drugs and cell types. The results have yielded some clue to the question, which we discuss in the present paper.

I. INTRODUCTION

Cytomagnetometry has been used to investigate mechanical properties of the cytoplasm for nearly 20 years. It was first conjectured by Nemoto[1] that the relaxation phenomenon observed in the magnetic field from the human lung which inhaled magnetic dust was due to the intracellular movements of the alveolar macrophages in the lung which randomized the direction of the magnetic moments of the particles within the cells which had been magnetized by an external magnetic field immediately before the relaxation measurement. The energy responsible for the relaxation process, which we designate as E_r , was also estimated by the method of secondary magnetization of the lung. Later, macrophages *in vitro* were used and it was shown that the relaxation process was indeed due to the intracellular movements within cells[2-4]. Since then several researchers have used this technique for studying some aspects of the biomechanics of cells [5-11]. Of recent interest is the study by Wang et al.[8] on the transduction of mechanical stimuli on the outer surface of the cell to the inner cytoskeletal structure.

In [1] the measurement method of E_r was proposed based on the simplest model in which a magnetic particle goes under rotational Brownian motion which is impeded by apparent viscosity of the cytoplasm. This model seemed to be enough for estimating E_r , although other experimental results showed that phagosomes containing the particles experience elasticity as well as viscosity and the randomizing force.

Models have been proposed for cytomagnetometry. The first model in [1] consisted of E_r and apparent viscosity η . Viscoelastic components were added to the model and the behavior of the model was examined in detail in [10]. Experimental data were treated with the viscoelastic model but in a somewhat simpler way in [11]. A different model using viscoelasticity was proposed but not discussed here.

In the present paper, we present our recent result with J774A.1 cells which showed intriguing behavior of E_r when MIA was added. MIA is known to inhibit the glycolysis

pathway in the ATP synthesis in cells. In the present paper, we present the results of the cytomagnetometric measurements and the microscopic observations of the cytoskeleton to discuss the strange behavior of E_r .

II. MODEL

1) *Energy E_r Responsible for Relaxation*: Cytomagnetometry is performed by applying a magnetic field to the cells which have engulfed magnetic particles and measuring the magnetic field from the cells. We simply call this field “cell field.” The cell field decays with the half time of 1-3 minutes due to the randomization of the directions of the magnetic moments of the phagosomes which are organelles within cells and contain the particles. Assume that all the phagosomes have the same strength of magnetization, the cell field normalized with respect to its value when all the moments are aligned is given by

$$B = \frac{1}{n} \sum_{k=1}^n \cos \theta_k = E[\theta] \quad (1)$$

where θ_k is the polar angle of the magnetic moment of the k -th phagosome, n the number of phagosomes containing particles, and $E[\cdot]$ is the mean. When an external magnetic field is applied to the ensemble of the magnetized phagosomes, they tend to align themselves in the direction of the applied field to decrease the potential energy $-\mu H \cos \theta$. The alignment is impeded by the randomizing energy E_r , which is considered to act like the thermal agitation kT . This association with statistical dynamics leads us to the Boltzmann distribution for the angle θ :

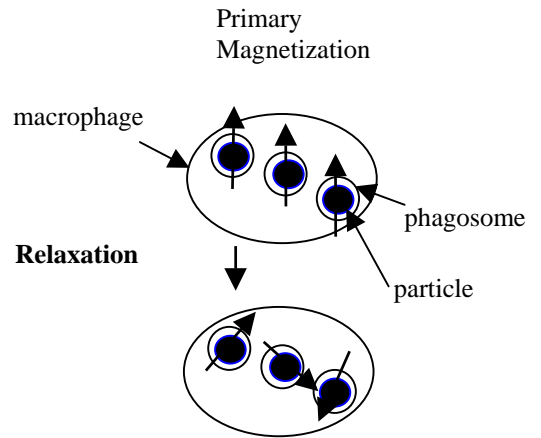


Fig. 1 Principle of cytomagnetometry.

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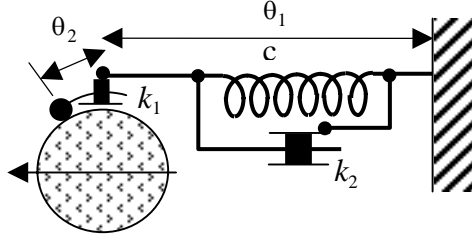


Fig. 2 Viscoelastic model of the impedance given to the phagosome.

$$p(\theta) = \exp\{\mu H \cos \theta / E_r\} \quad (2)$$

From this we can get the Langevin function

$$L(a) = \coth a - 1/a, \quad a = \mu H / E_r \quad (3)$$

for the cell field in equilibrium under the external field H . Here μ is the magnetic moment of the phagosome (particle).

2) *Viscoelastic Model* Fig. 2 shows the viscoelastic model of the tissues surrounding the phagosome. It consists of three elements; k_1, k_2 are viscous elements and c represents elasticity. The differential equations governing the dynamics are :

$$\begin{aligned} \frac{d\theta_1}{dt} &= -\frac{c}{k_2}(\theta_1(t) - \tilde{\theta}_1) + \frac{1}{k_2} \left(\frac{dW(t)}{dt} + f(t, \theta) \right) \\ \frac{d\theta_2}{dt} &= \frac{1}{k_1} \left(\frac{dW(t)}{dt} + f(t, \theta) \right) \end{aligned} \quad (4)$$

where θ_1 and θ_2 are defined as in Fig. 2 and $\theta = \theta_1 + \theta_2$ is the direction of the magnetic moment of the phagosome with respect to that of the measurement. $\tilde{\theta}_1$ is the natural length of the elastic body. $f(t, \theta)$ is the torque exerted by the external magnetic force. $W(t)$ is the one-dimensional Brownina motion with mean and variance given by $E[W(t)] = 0$, $V[W(t)] = \alpha t$ where $\alpha > 0$.

Assume that a strong pulse magnetic field is given to the cells which magnetizes the paricles in its direction. The pulse magnetizing field does not mechanically rotate the phagosomes. It is shown [10] that the variance of θ after pulse magnetization ($f(t, \theta) = 0$) is given by

$$V[\theta(t)] = \alpha \left\{ \frac{1 - e^{-\sigma t}}{\sigma k_2^2} + \frac{2(1 - e^{-\sigma t})}{\sigma k_1 k_2} + \frac{1}{k_1^2} \right\} \quad (5)$$

where $\sigma = c/k_2$. Since $E[\theta(t)] = 0$ and θ is normally distributed, the cell field normalized with respect to its maximum value is given by

$$B(t) = \frac{1}{\sqrt{2\pi V[\theta]}} \int \cos \xi \exp \left\{ -\frac{\xi^2}{2V[\theta]} \right\} d\xi = \exp \left\{ -\frac{V[\theta]}{2} \right\} \quad (6)$$

Consideration of the model suggests that

$$\alpha = 2E_r \kappa, \quad \kappa = (k_1^{-1} + k_2^{-1})^{-1} \quad (7)$$

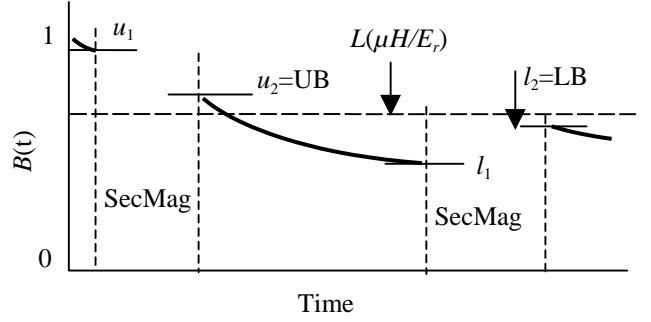


Fig. 3 Method to obtain upper and lower bounds for the cell field in equilibrium under external field H .

would be a good choice for the relationship between these values[10].

III. METHODOLOGY

1) *Preparation of Cells and Particles*: We used both cultured cells called J774A.1 and hamster alveolar macrophages (hereafter abbreviated as AlvMph). About 0.5 million cells were incubated in a bottle of diameter 12mm with $8\mu\text{g}$ of spherical Fe_3O_4 particles of diameter $\sim 1.2\mu\text{m}$ produced by Moeller[7]. Most of the particles were phagocytized in several hours.

2) *Cytomagnetometry*: The magnetometer is a flux-gate device by Institut Dr. Foerster (1.068). The bottle containing the cells was rotated at 4 rpm between the pair of probes for signal detection. The RMS value of the noise was $1 - 2 \times 10^{-11}$ T. The ambient temperature was controled within $37 \pm 1^\circ\text{C}$.

For relaxation measurement, the sample was magnetized by a pulse field of $\sim 0.3\text{T}$ and $< 1\text{ms}$ duration and relaxation curve was measured for 7 miutes. For E_r measurement, two measurements were made to determine an upper bound (UB) and a lower bound (LB) for the Langevin function value $L(a)$, the cell field in equilibrium under the external field H . See Fig. 3. $L(a)$ was estimated by the linear extrapolation :

$$L(a) = \frac{u_1 l_2 - u_2 l_1}{u_1 + l_2 - u_2 - l_1} \quad (8)$$

3) *Microscopic Observations of Cytoskeleton* To correlate cytomagnetometric measurement with cytoskeletal structure changes after treatment with drugs, we observed micro-filaments and microtubules by a scanning laser confocal microscope equipped with argon laser(Biorad MRC600). To visualize microtubules, the cells adhered to a cover slip were fixed with 0.5% solution of glutaraldehyde and first treated with mouse anti β -tubulin and then with FITC-conjugated anti mouse igG. The cells were stained for microfilaments with FITC-labeled phalloidin. The detail of the method for the immuno-fluorescent microscopy is ommited in the present paper.

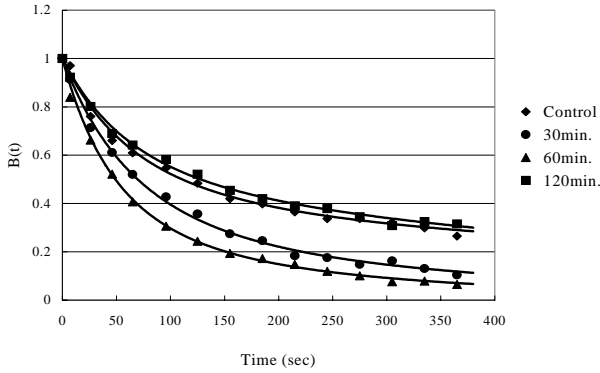


Fig. 4 Relaxation curves from J774A.1 after treatment with MIA.

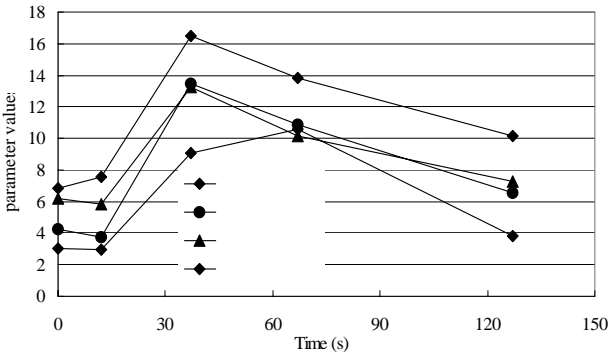


Fig. 5 The change of parameter values after treatment with MIA .

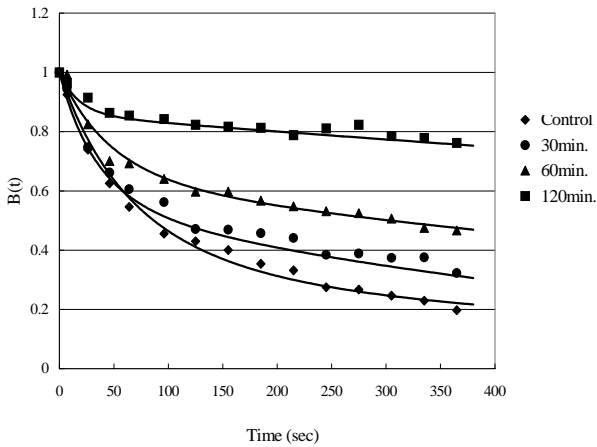


Fig. 6. Relaxation curves from alveolar macrophages after MIA treatment.

4) *Treatment with Drugs* In the previous study[1], KCN was used to inhibit ATP synthesis in hamster AlvMph. We knew from our previous experiment that KCN little affected J774 cells and hence, MIA was used. It is known to inhibit the glycolysis process in ATP synthesis whereas KCN inhibits oxydative phosphorylation path. Drugs affecting

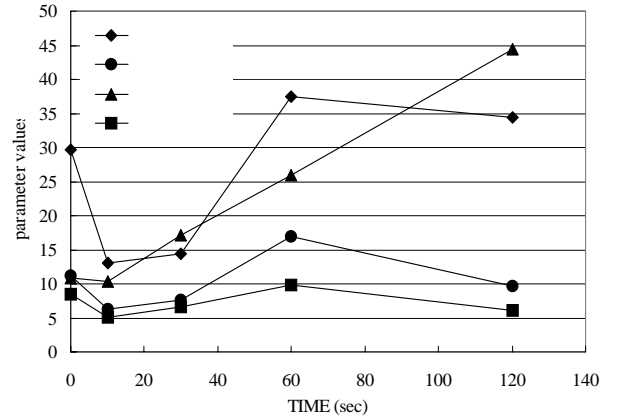


Fig. 7. Parameter value changes after MIA treatment for alveolar macrophages.

cytoskeletal filamnts were also used: cytochalasin to destroy microfilaments and colchicine to destroy microtubules.

IV. RESULTS

1) *Cytomagnetic Measurements* Fig. 4 shows four relaxatkon curves for J774A.1 cells before and after treatment with MIA. Although they are intended to show the time course of the change of the relaxation curve, each bottle of cells was used for a single relaxation measurement only. The generous use of the cells was necessary because the behavior of the relaxation curve depended significantly on how many times the cells had been used. At 30-60 minutes after treatment, the relaxation became significantly faster than the control curve. At 120 minutes it became slower than the control curve.

Fig. 5 shows the change of the parameter values with the time which elapsed after treatment with MIA. Each point in the curves represents the average of 3-5 experiments. It is seen that E_r increased 2 to 3 -fold from the control value. Although it decreased from 60 to 120 minutes, it was still larger than the control value. The other parameter values behaved much like E_r . Fig. 6 shows the change of the relaxation measured in hamster AlvMph after treatment with MIA. In this case the longer the time after treatment, the slower the relaxation became. Fig. 7 shows the change of the parameter values after MIA treatment correponding to Fig. 6.

2) *Cytoskeletal Change Optically Observed* Fig. 8 shows images of microfilaments in J774A.1 cells under control condition and 60 min after treatment with MIA. It is seen that the ATP deficiency caused by MIA destroyed microfilaments. The longer the time elapsed, the more the destruction proceeded. Fig. 9 shows the response of microtubules to MIA. The effect is much less noticeable than in the case of microfilaments. However, peripheral areas seem to be somewhat disturbed.

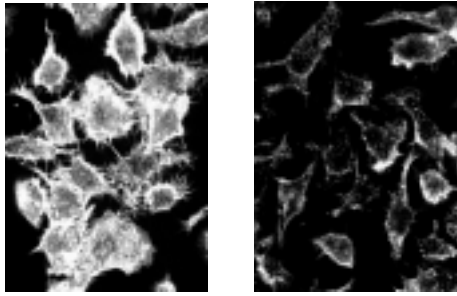


Fig. 8 Microfilaments before (left) and 60 minutes after (right) MIA treatment. J774A.1 cells.

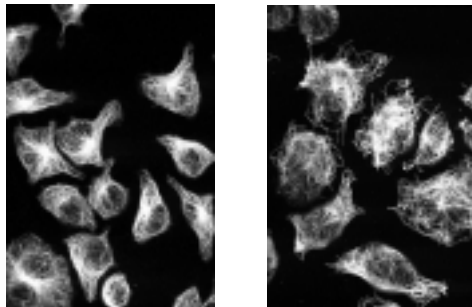


Fig. 9 Microtubules before (left) and 60 min after (right) MIA treatment.

V. DISCUSSION

1) *Increase of E_r after MIA Treatment* Fig. 4 shows that relaxation became faster after MIA treatment and Fig.5 shows that E_r became greater at the same time. This was quite contrary to our expectation that the decrease in ATP concentration should cause slower relaxation due to decrease in E_r , which is exactly what happened with AlvMph treated with KCN [6]. Fig. 8 showing E_r of AlvMph treated with MIA reveals a slight decrease in E_r during the first 10 minutes but on the whole E_r did not change significantly. The relaxation became very much slower as shown in Fig. 7, which then had to be attributed to increased k_1 and c . Although E_r did not increase as in the case of J774A.1, its relatively constant time course after MIA treatment is still intriguing like the case of J774A.

2) *Microscopic Observations and E_r* MIA significantly reduced microfilaments in J774A.1 cells. This is consistent with the well known fact that forming microfilaments (F-actin) from G-actin molecules requires ATP. On the other hand, microtubules do not need ATP for polymerization from tubulin monomers. Microtubules after MIA treatment (Fig. 10) shows that they are somewhat disturbed, which is probably due to the loss of anchorage formed by connections with microfilaments. It is therefore possible that some kind of potential energy stored in the elasticity of microtubules is relaxed when microfilaments are destroyed by MIA and this energy counts for the increase of E_r and the faster relaxation. This is merely a speculation and we have to look for more possibilities and find the true mechanism.

VI. CONCLUSION

J774A.1 cells and hamster AlvMph were used for cytomagnetometry to measure the energy E_r responsible for the relaxation of cell field. E_r increased in J774A.1 cells when treated with MIA and did not decrease in AlvMph cells treated likewise. It is an interesting phenomenon considering that ATP deficiency caused by MIA would most likely to decrease E_r . It may be attributed to the course of cytoskeletal change after disappearance of microfilaments by MIA treatment.

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